TP53 Polymorphism of Exon 4 at Codon 72 in Cutaneous Squamous Cell Carcinoma and Benign Epithelial Lesions of Renal Transplant Recipients and Immunocompetent Individuals: Lack of Correlation with Human Papillomavirus Status[1]

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A common polymorphism at codon 72 of exon 4 encoding either arginine or proline has been shown to confer a susceptibility to the development of skin tumor in renal transplant recipients. Moreover, this polymorphism may affect proteolytic degradation of p53 promoted by E6 protein from mucosal human papillomaviruses and represent a risk factor for human-papillomavirus-induced carcinogenesis. In this study, we analyzed the human papillomavirus presence and the TP53 allele distribution in cutaneous squamous cell carcinoma of renal transplant recipients and immunocompetent patients. Fifty-three squamous cell carcinomas from 40 renal transplant recipients, 50 benign epithelial skin lesions from 50 renal transplant recipients with no history of skin cancer, 51 squamous cell carcinomas from immunocompetent patients, and 29 blood samples from immunocompetent individuals without skin cancer were investigated. Human papillomavirus DNA was detected using polymerase chain reaction performed with two pairs of primers (MY09–MY11 and FAP59–FAP64). TP53 allele distribution was studied by denaturing gradient gel electrophoresis assay, followed by sequencing analysis. Human papillomavirus DNA was detected in 64% of squamous cell carcinoma and 79% of benign epithelial lesions from renal transplant recipients (NS) and only in 37% of squamous cell carcinoma from immunocompetent patients ($p < 0.05$). Mucosal oncogenic human papillomavirus types were predominant in squamous cell carcinoma from both renal transplant recipients and immunocompetent patients. Rate of arginine homozygosity in squamous cell carcinoma from renal transplant recipients was significantly higher (83%) than in immunocompetent patients with or without squamous cell carcinoma (60% and 59%, respectively). Our results suggest that TP53 arginine/arginine genotype could represent a potential risk factor for the development of squamous cell carcinoma in renal transplant recipients compared to immunocompetent patients. No association between TP53 arginine/arginine genotype and human papillomavirus status could be determined, however. ***Key words: human papillomavirus/p53 polymorphism/renal transplant recipient/squamous cell carcinoma. J Invest Dermatol 118:1026–1031, 2002***

Renal transplant recipients (RTR) are at an increased risk of developing nonmelanoma skin cancer and the cumulative risk is 30%–60% 20 y after transplantation, depending on geographic differences and ethnic origin (Boyle *et al*, 1984; Blohme and Larko, 1990; Hardie, 1995; Barba *et al*, 1996; Lindelöf *et al*, 2000). Squamous cell carcinomas (SCC) are the most prevalent, although the frequency of basal cell carcinoma and malignant melanoma is also increased. There are several specific factors that may explain the susceptibility to skin cancer in RTR. The most important risk factors are skin type, pretransplant and postransplant sun exposure (Boyle *et al*, 1984), duration of immunosuppressive treatment (Ducloux *et al*, 1998), immunogenetic factors linked to HLA class II antigens (Bouwes Bavinck *et al*, 1997), and infection with human papillomaviruses (HPV) (Bouwes Bavinck and Berkhout, 1997). The high prevalence of HPV DNA detected in SCC from RTR suggests a potential role for HPV infection in the etiology of these tumors (Burk and Kadish, 1996). The types of HPV that are possibly implicated are discussed as a wide diversity of HPV can be detected within a single SCC, including mucosal HPV (types 6, 11, 16, and 18), epidermodysplasia verruciformis (EV) related subgroup (types 5, 8, etc.), and other HPV types (Tieben *et al*, 1994;

Manuscript received August 10, 2001; revised February 5, 2002; accepted for publication February 14, 2002.

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Abbreviations: Arg, arginine; BCL, benign cutaneous lesion; DGGE, denaturing gradient gel electrophoresis; EV, epidermodysplasia verruciformis; ICP, immunocompetent patients; LOH, loss of heterozygosity; Pro, proline; RTR, renal transplant recipients.

[1]This work was presented in part at the 19th International Papillomavirus Conference in Florianopolis, Brazil, September 1–7, 2001.

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Table I. HPV types detected in SCC or BCL from RTR and ICP. Mucosal HPV were detected using MY09–MY11 PCR primers and cutaneous HPV with FAP59–FAP64 PCR primers

	% mucosal HPV	% cutaneous HPV	% coinfection	Total
1- RTR + SCC (53)	58.5 (31)	24.5 (13)	19 (10)	64 (34)
2- RTR + BCL (38)[a]	66 (25)	26 (10)	13 (5)	79 (30)
3- ICP + SCC (51)	29 (15)	12 (6)	4 (2)	37 (19)
Statistical analysis				
1 *vs* 2	NS	NS	NS	NS
1 *vs* 3	p = 0.003	NS	p = 0.017	p = 0.006
2 *vs* 3	p = 0.001	NS	NS	p = 0.001

[a]38 samples of 50 BCL were analyzed for HPV infection.

Table II. Frequency of mucosal HPV types detected in SCC from RTR and ICP

HPV	untyped	6/11	16	18	35	45	58	Total
RTR + SCC	6.5	0	51.6	29	3.2	3.2	6.5	100
(*n* = 31)	(2)	0	(16)	(9)	(1)	(1)	(2)	
ICP + SCC	0	0	66.6	26.7	0	0	6.7	100
(*n* = 15)	0	0	(10)	(4)	0	0	(1)	

Shamanin *et al*, 1996; Hopfl *et al*, 1997; de Jong-Tieben *et al*, 2000). The carcinogenic effect of HPV may be explained in part by the transforming viral protein E6, which binds to and induces the degradation of p53 through the ubiquitin pathway (Scheffner *et al*, 1990), and inhibits apoptosis in response to ultraviolet irradiation through other unexplained mechanisms (Jackson and Storey, 2000). The equally high prevalence of EV-HPV infection in RTR with or without a history of skin cancer (de Jong-Tieben *et al*, 2000), however, suggests that, besides HPV infection, other factors may play a critical role. Among them, genetic factors predisposing some infected patients might account for different individual susceptibilities in the development of skin cancer. A common polymorphism of the tumor suppressor gene TP53 that results in either a proline (Pro) or arginine (Arg) at residue 72 of exon 4 has been described (Matlashewski *et al*, 1987). The 72Arg form of the p53 protein appears to be particularly susceptible to HPV16, HPV18, and HPV11 E6-associated degradation *in vitro* (Storey *et al*, 1998), and could facilitate the oncogenic effect of HPV infection. In addition, it has been demonstrated that the Arg-encoding allele may represent a significant risk factor in the development of SCC in RTR (Storey *et al*, 1998). This finding was not confirmed by a recent report (Marshall *et al*, 2000*b*), however, and the importance of TP53 polymorphism in HPV-associated tumors is still a matter of controversy (Dokianakis *et al*, 2000; Bastiaens *et al*, 2001; O'Connor *et al*, 2001). To gain insight into the role of genetic variation of TP53 in skin carcinogenesis, we analyzed the HPV presence and the TP53 allele distribution in cutaneous SCC and benign epithelial lesions of RTR and immunocompetent patients (ICP).

MATERIALS AND METHODS

Study population Only Caucasian French RTR or ICP were included in the study to avoid confounding factors due to ethnic differences, and informed consent was obtained. Fifty-three SCC from 40 different RTR, 50 benign cutaneous epithelial lesions (BCL) from 50 RTR, and 51 SCC from 51 ICP were included along with blood samples from 29 healthy controls. The mean duration of immunosuppression was similar in both groups of RTR (96 mo ± 69 *vs* 76 mo ± 60). All SCC from both RTR and ICP were located on sun-exposed areas. As expected, the mean age at presentation of SCC was significantly lower in RTR (63 y) than in ICP (77 y), and the age of patients with BCL was 46 y. We also tested blood samples from nine RTR presenting SCC to analyze loss of heterozygosity (LOH).

DNA extraction Pathologic tissues (BCL and SCC) were fixed either with Bouin or buffered formalin, and embedded in paraffin. Tumoral cells were separated from normal surrounding tissues by microdissection. Ten serial 5 μm paraffin-embedded sections were used for DNA extraction from these tissues as follows. Paraffin was removed twice with xylene (1 ml) and twice with absolute ethanol (1 ml). The pellet was air-dried, resuspended in 100 μl of digestion buffer [50 mM Tris pH 8, 0.1 mM ethylenediamine tetraacetic acid (EDTA), 0.5% Tween-20, 200 μl per ml proteinase K], incubated overnight at 37°C, and then boiled for 5 min. The DNA from blood samples was extracted using the Quiagen kit as described by the manufacturer (Quiagen, France).

HPV genotyping After DNA extraction, the presence of HPV DNA was tested by polymerase chain reaction (PCR) using two different sets of L1 open reading frame consensus primers. MY09–MY11 primers allowed the production of 450 bp amplicons and the detection of a broad spectrum of mucosal HPV (Manos *et al*, 1989). The PCR products were then typed using the Hybridowell kit, as we previously described (Riethmuller *et al*, 1999). To genotype the most common HPV, the amplimers were hybridized with internal 5′ biotinylated probes for low risk HPV 6, 11, or high risk HPV 16, 18, 31, 33, 35, 45, 51, 52, 58, 68. The primers FAP59–FAP64 generated amplicons of 480 bp and amplified not only a broad range of cutaneous HPV, including EV-associated HPV types, but also mucosal HPV types (Forslund *et al*, 1999).

PCR of TP53 exon 4 and denaturing gradient gel electrophoresis (DGGE) For DGGE analysis, TP53 exon 4 was amplified with consensus 40nGC-clamped primers TP53.4.1F–R that allowed the production of a 224 bp fragment (Hildesheim *et al*, 1998). The primer sequences were as follows: TP53.4.1F, 5′-[40GC]CCTGGTCCTCTG-ACTGCTCT-3′; TP53.4.1R, 5′-GTGTAGGAGCTGCTGGTGCA-3′. The amplification was carried out in a 100 μl volume containing 1 × PCR buffer (Perkin), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 2.5 U *Taq* DNA polymerase (Ampli *Taq*, Perkin Elmer), 25 pmol of each primer, and 1 μg of template DNA. After DNA denaturation, 35 cycles consisting of 30 s at 94°C, 15 s at 60°C, 20 s at 72°C were performed, followed by a 7 min final extension at 72°C. The PCR products were then analyzed by DGGE in the following conditions. A 16 × 18 cm, 1 mm thick, 8% acrylamide/Bis (37.5:1) gel with a parallel denaturing gradient range of 35%–75% in 1 × TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) was used. The gradient gel was cast using Bio-Rad's Model 475 gradient delivery system. Forty microliters of PCR products were mixed with 5 μl of 2 × gel loading and electrophoresed on the Dcode system. The gel was then stained with ethidium bromide in 1 × TAE buffer for 5 min and visualized under ultraviolet transillumination. The DGGE analysis of 10 SCC from RTR, eight SCC from ICP, and nine blood samples from RTR presenting SCC was confirmed by automatic sequencing, after purification of their PCR products (224 bp). Samples were subjected to cycle sequencing with the forward primer TP53.4.1F without the 40nGC clamp using the CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter) following the cycling conditions: 20 s at 96°C, 20 s at 50°C, and 4 min at 60°C for 30 cycles ended by holding at 4°C.

Statistical analysis Pearson χ^2 test with Yates's correction when necessary was carried out using Systat software to test the differences of polymorphism according to virologic results. Statistical significance was considered at $p < 0.05$.

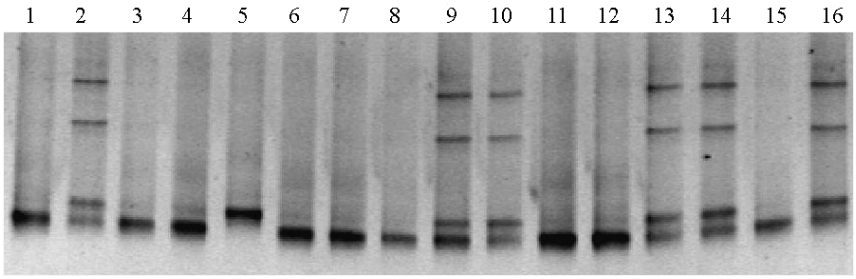

Figure 1. Exon 4 DGGE analysis of TP53 gene in SCC. *Lane 1*: DNA from C33A cell control homozygous Arg/Arg. *Lane 2*: DNA from CaSki cell control heterozygous Arg/Pro. *Lanes 3*, *4*, *6*, 7, *8*, *11*, *12*, *15*: DNA from SCC samples homozygous Arg/Arg. *Lanes 9*, *10*, *13*, *14*, *16*: DNA from SCC samples heterozygous Arg/Pro. *Lane 5*: DNA from SCC samples homozygous Pro/Pro.

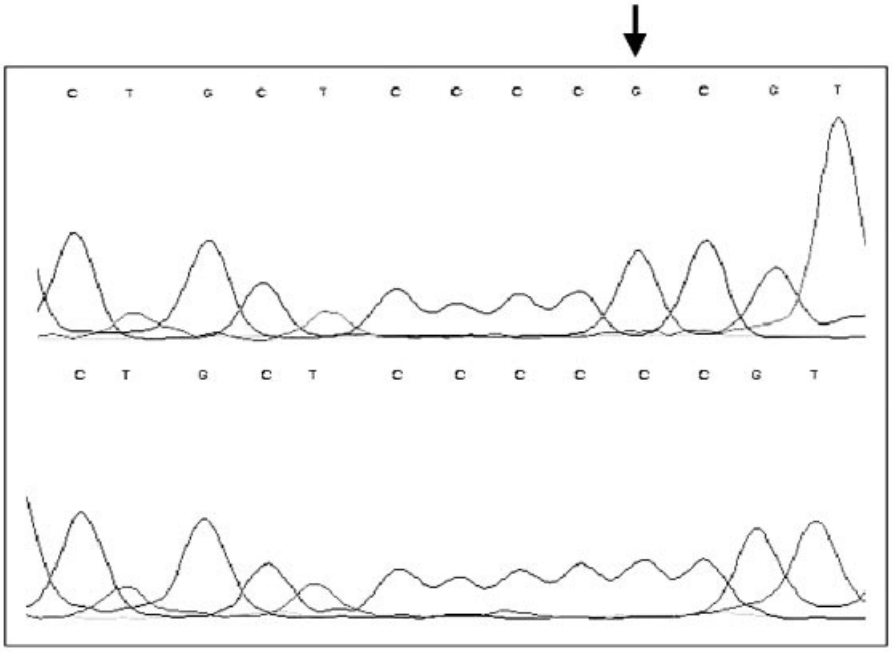

Figure 2. Genotypic distribution of TP53 gene. Sequencing of p53 codon 72. The figure shows chromatograms from the CEQ2000 automat sequencer. The letters above the peaks represent the appropriate base. The arrow indicates the codon 72 polymorphism. At the top, the sequence of tumor DNA from RTR + SCC presenting a CGC (Arg) at codon 72 is shown. At the bottom, the sequence DNA from RTR + SCC presenting a CCC (Pro) at codon 72 is shown.

RESULTS

HPV status The overall prevalence of HPV is shown in **Table I**.

Among BCL, 38 of 50 specimens were analyzed for HPV infection, because β-globin DNA could not be amplified in the remaining specimens. Lack of β-globin DNA detection probably resulted from disintegrated DNA probably linked to the Bouin fixation procedure. HPV DNA was detected in 64% of SCC and 79% of BCL from RTR ($p = 0.12$), and only in 37% of SCC from ICP ($p = 0.006$). In SCC from RTR, significantly more mucosal HPV were present (58.5%) than cutaneous HPV (24.5%) ($p = 0.001$). Among mucosal HPV, HPV 16 and 18 predominated in SCC of both RTR and ICP (**Table II**). In addition, coinfection (mucosal and cutaneous HPV) was present in 10 SCC from RTR (19%). It was notable that the ratio of positive cases for mucosal HPV *versus* cutaneous HPV was similar in all groups of patients, i.e., 2.4–2.5. This suggested that, although the prevalence of HPV infection increased in RTR with SCC *versus* RTR with BCL *versus* ICP with SCC, the ratio of mucosal HPV to cutaneous HPV remained similar.

Genotypic distribution of TP53 gene **Figure 1** shows the representative results of DGGE analysis with three band shift patterns. Single bands corresponded to homozygous p53 codon 72 genotypes, either Arg/Arg or Pro/Pro, whereas four bands attested to a heterozygous genotype. After sequencing analysis of the single bands, we confirmed that the upper band corresponded to a Pro genotype and the lower one to an Arg genotype (**Fig 2***).*

Our test was validated on CaSki cells (Arg/Pro) and C33A cells (Arg/Arg). Among SCC from RTR, 35 were genotyped and compared to 35 SCC from ICP and 29 blood samples from healthy controls. The polymorphism of TP53 was not studied in BCL because of insufficient material. The proportions of p53 codon 72 genotype found were 83% Arg homozygous, 3% Pro homozygous, and 14% Arg/Pro heterozygous in RTR with SCC, compared to 60% Arg homozygous, 9% Pro homozygous, and 31% Arg/Pro heterozygous in ICP with SCC (**Table III**).

Statistical analysis showed that the homozygosity rate of Arg in RTR with SCC was significantly higher than in ICP with SCC ($p = 0.034$). In addition, there were no significant differences in the distribution of the homozygous p53 Arg allele between ICP with or without SCC (60% *vs* 59%). To verify the absence of LOH, TP53 genotype at codon 72 was analyzed in nine SCC from RTR and in nine blood samples from the same patients as described previously. In all cases the TP53 genotype was similar both in the SCC and in the blood sample, confirming the absence of LOH.

Table III. Proportional distribution of TP53 genotype in SCC from RTR and ICP

	% Arg/Arg	% Arg/Pro	% Pro/Pro
1- RTR + SCC (35)[a]	83 (29)	14 (5)	3 (1)
2- ICP + SCC (35)[a]	60 (21)	31 (11)	9 (3)
3- ICP (29 blood samples)	59 (17)	24 (7)	17 (5) (29 blood samples)
Statistical analysis			
1 *vs* 2	$p = 0.034$		
1 *vs* 3	$p = 0.032$		
2 *vs* 3	NS		

[a]35 patients of 40 RTR + SCC, 35 patients of 51 ICP + SCC, and 29 blood samples from ICP were analyzed for TP53 genotype.

Correlation between the presence of HPV and the p53 codon 72 Arg polymorphism The distribution of codon 72 TP53 genotype with respect to HPV status demonstrated similar prevalences of Arg/Arg, Arg/Pro, and Pro/Pro in HPV-negative samples and in HPV-infected patients. The statistical analysis did not indicate any significant association between HPV status and TP53 polymorphism.

DISCUSSION

This study was conducted to compare the HPV status and TP53 codon 72 polymorphism in SCC and BCL from immunosuppressed RTR and in SCC from ICP. The blood samples of a reference population of 29 healthy blood donors were used as controls for TP53 codon 72 polymorphism.

With two pairs of HPV primers (MY09–MY11 and FAP59–FAP64), we were able to detect a high rate of viral DNA in lesions from RTR with no significant differences in prevalence between SCC and BCL (64% *vs* 79%) and between HPV types, either mucosal (58.5% *vs* 66%) or cutaneous HPV (24.5 *vs* 26%). In SCC lesions from ICP, the overall HPV presence was lower ($p < 0.05$). In addition, mixed mucosal and cutaneous HPV types were mostly detected in lesions from RTR, either SCC or BCL.

It was interesting to note that the ratio of positive cases for mucosal HPV *versus* cutaneous HPV was similar in all groups. This suggested that, although the prevalence of HPV infection increased in RTR with SCC *versus* RTR with BCL *versus* ICP with SCC, the ratio of mucosal HPV to cutaneous HPV remained similar. To our knowledge this has never been previously noted. The different studies on HPV infection in SCC from RTR are difficult to compare, however, as different primers either for cutaneous HPV or for mucosal HPV were used. In our study, the MY09–MY11 degenerate PCR system followed by typing with biotin-labeled probes was successful in identifying, at a high rate, oncogenic mucosal HPV types 16, 18. This is in agreement with the data obtained by Euvrard *et al* (1993). Discrepancies with other studies, which detected mostly EV or EV-related viruses (Berkhout *et al*, 1995), are likely to reflect the differing profiles of the PCR primers used. For identification of cutaneous HPV, a general PCR method using FAP59–FAP64 primers has recently been described (Forslund *et al*, 1999). Such a method allows the detection of a large HPV spectrum, including EV-related HPV, other HPV types (3, 10, 25, 27, 28, 29, 77, etc.), which are phylogenetically grouped with the mucosal HPV types, and also low risk (6, 11, 42) or high risk (16, 18, 31, 52, 58, 68) mucosal HPV types. The involvement of specific HPV types in promoting skin carcinogenesis cannot be ruled out, as amplicons generated by that PCR have not been sequenced in our study. The frequency of HPV DNA in lesions (either benign or malignant) from ICP (37%) was significantly lower than in lesions from RTR (79% and 64%, respectively). It is likely that immunosuppression may play a role in increasing susceptibility to HPV infection in RTR. Other cofactors (i.e., sun exposure or genetic variation) may also be involved in viral replication, however.

It has been demonstrated that HPV alters TP53 stability. Indeed, HPV E6 oncoprotein binds to p53 and induces its degradation through the ubiquitin pathway (Scheffner *et al*, 1990). A recent report (Storey *et al*, 1998) suggested that a common polymorphism at codon 72 of the TP53 tumor suppressor gene might be a risk factor in the development of HPV-associated cancers. The previously suggested association between TP53 codon 72 Arg homozygosity and SCC from RTR (Storey *et al*, 1998) is confirmed in our much larger study. Indeed, Storey *et al* (1998) studied 32 HPV-infected SCC from 12 RTR and demonstrated a striking excess of patients with only Arg (75%), rather than Arg/Pro (25%) or Pro (0%) alleles. The relationship between TP53 status and the presence of HPV in SCC was not established, and ICP with and without SCC were not compared. In our study (**Table III**), we analyzed 35 SCC from 40 RTR and found a strong prevalence of Arg homozygosity (83%) compared to Arg/Pro heterozygosity (14%) or Pro homozygosity (3%). In contrast, the rate of Arg homozygosity in 35 SCC from ICP and in 29 leukocyte DNA from healthy subjects was significantly lower – 60% and 59%, respectively. Our data indicate that the TP53 codon 72 Arg allele may confer susceptibility to the development of SCC after renal transplantation. This contrasts with a previous study conducted with a cohort of long-term survivors of renal transplantation (Marshall *et al*, 2000*b*). The authors used leukocyte DNA for detection of TP53 polymorphism and observed a 53% rate of Arg homozygosity in 34 RTR with SCC, similar to rates of Arg homozygosity in 188 RTR without SCC (53%) and in 84 controls (46%).

When focusing on ICP with SCC or without SCC, our results are in agreement with those obtained in previous studies (Hamel *et al*, 2000; Bastiaens *et al*, 2001; O'Connor *et al*, 2001). The distribution of the TP53 codon 72 genotype (Arg/Arg, Arg/Pro, Pro/Pro) was similar in both groups: 60%, 32%, and 8% *vs* 59%, 24%, and 17%. Similar results were observed in recent reports (Hamel *et al*, 2000; Bastiaens *et al*, 2001; O'Connor *et al*, 2001), and the authors concluded that TP53 codon 72 Arg homozygosity does not appear to represent a significant risk factor for cutaneous SCC in ICP. These results were not confirmed by Dokianakis *et al* (2000) in a study conducted on 29 high-risk HPV-related skin lesions compared to blood samples from 61 healthy individuals. The low rate of TP53 Arg/Arg (20%) in the control group, however, compared with the frequency of TP53 codon 72 Arg homozygosity (40%–80%) observed in most studies (Storey *et al*, 1998; Hamel *et al*, 2000; Marshall *et al*, 2000*b*; Bastiaens *et al*, 2001; O'Connor *et al*, 2001) should be questioned. It has been proven that p53 polymorphism varies according to geographic origin, and the prevalence of TP53 Pro allele is closely related to latitude and increases when approaching the Equator (Beckman *et al*, 1994). In our study, we genotyped a well-established cohort of 40 RTR with comparable immunosuppressive treatments. Although the lack of information regarding skin type remains a possible confounding variable, all individuals were of European Caucasoid origin (Eastern France). Our study involves the first French series of patients tested for p53 polymorphism and gives new information on the distribution of TP53 polymorphism in a homogeneous population.

Table IV. Potential factors involved in the etiology of SCC

Factors	RTR	ICP
Immunosuppression	+ + + +	0
HPV infection	+ + + +	+ +
TP53 codon 72 Arg genotype	Yes	No
Duration of UV exposure[a]	+ +	+ + + +
Other	?	?

[a]Duration of sun exposure in RTR was supposed to be lower than in ICP, as the age at presentation of SCC was significantly lower in RTR (63 y *vs* 77 y).

Another potential source of bias with previous studies (Storey *et al*, 1998; Dokianakis *et al*, 2000; Hamel *et al*, 2000; Marshall *et al*, 2000*b*; Bastiaens *et al*, 2001; O'Connor *et al*, 2001) is that genotyping was not performed on similar material. For detection of TP53 polymorphism, tumor material as well as leukocyte DNA can be used. In our study, we evaluated TP53 polymorphism in skin tumors from RTR and ICP, and in leukocyte DNA from ICP without SCC. Storey *et al* (1998) performed TP53 genotyping on DNA derived from SCC, whereas leukocyte DNA was used in other studies (Dokianakis *et al*, 2000; Hamel *et al*, 2000; Marshall *et al*, 2000*b*; Bastiaens *et al*, 2001; O'Connor *et al*, 2001). In addition, the use of tumor material for detection of TP53 polymorphism may be associated with the risk that LOH interferes with our results. Indeed, LOH is frequently observed in skin cancer on chromosome 17p, where TP53 is located (Quin *et al*, 1994). Although Storey *et al* (1998) demonstrated that LOH was not an important mechanism for overrepresentation of TP53 Arg genotype in cervical HPV-related cancer, at least two of the 12 RTR included in the same study showed LOH in the SCC. Our study can potentially eliminate such a bias, as we found the same genotype after testing TP53 polymorphism on DNA from nine tumor specimens compared to leukocyte DNA in the same patients.

Few studies determined the relation between HPV infection and TP53 codon 72 polymorphism. We did not observe any association between TP53 Arg/Arg genotype and HPV status as demonstrated in two previous reports (Bastiaens *et al*, 2001; O'Connor *et al*, 2001). Storey *et al* (1998) and Dokianakis *et al* (2000) demonstrated a high frequency of TP53 Arg homozygosity in HPV-associated skin lesions, but no HPV-negative lesions or HPV-associated controls were tested.

Altogether, our results indicate a significant association between TP53 codon 72 Arg homozygosity and cutaneous SCC in RTR, but not in ICP with SCC. Thus, the TP53 codon 72 Arg allele appears to be particularly important in immunosuppressed patients who undergo cumulative effects of long-term immunosuppression and increased susceptibility to HPV infection. Although the reason is not clear, we may suggest that immunosuppression increases susceptibility to HPV infection, particularly oncogenic mucosal HPV types 16, 18, and 45 as we showed. Subsequently, HPV may exert a carcinogenic effect by the transforming viral protein E6, which induces the degradation of tumor suppressor protein p53 (Scheffner *et al*, 1990) and inhibits apoptosis in response to ultraviolet radiation (Jackson and Storey, 2000). Thus, the cumulative effect of immunosuppression, oncogenic HPV infection, and TP53 codon 72 Arg homozygosity may explain the increased prevalence of HPV-infected SCC in RTR patients. There is also evidence against HPV E6 having a pathogenic role with respect to p53 inactivation in SCC (McGregor *et al*, 1997), however, as ultraviolet-related TP53 mutations are implicated in post-transplant skin cancer irrespective of HPV status. In addition, Marin *et al* (2000) showed that a common TP53 polymorphism at codon 72 of exon 4 influences the ability of certain p53 mutants to form stable complexes with p73 protein. Formation of such complexes correlates with a loss of p73 DNA-binding capability, and consequently its ability to serve as a sequence-specific transcriptional activator of TP53 and an inducer of apoptosis. The authors demonstrated that the ability of mutant p53 to bind p73, neutralize p73-induced apoptosis, and transform cells was enhanced when codon 72 of TP53 gene encoded Arg. In addition, the Arg-containing allele was always preferentially mutated in nonmelanoma skin cancer and all p53 mutants bound to p73. These data suggest that codon 72 plays a major role in skin carcinogenesis irrespective of HPV infection. Other studies, however, have shown that p73 may be inactivated by both high- and low-risk HPV E6, independently of ubiquitin-mediated proteolysis (Park *et al*, 2001), and that expression of p73 is driven by E2F-1 (Irwin *et al*, 2000; Lissy *et al*, 2000). Furthermore, O'Nions *et al* (2001) have suggested that E7 proteins in HPV-positive tumors may be associated with pRb and deregulate expression of E2F-1 and correspondingly p73. These observations strongly suggest that p53 homolog p73 may also have a role in skin carcinogenesis, and that interaction between this homolog and HPV may be relevant. Its role in immunocompromised patients remains to be elucidated, however. Moreover, intratype variation in the E6 gene of HPV 16 has been recently suggested as a possible additional factor in determining the susceptibility to cervical cancer associated with the TP53 Arg isoform (van Duin *et al*, 2000). As our data showed that oncogenic mucosal HPV types 16, 18, and 45 predominated in SCC of RTR, we may suggest that an association of TP53 codon 72 Arg/Arg genotype and infection by such viruses may confer a high risk of SCC development in RTR (**Table IV**). In addition, other cofactors such as ultraviolet radiation and related genetic variations (Marshall *et al*, 2000*a*) and other transforming or mutagenic properties of E6 or other viral proteins (Jackson and Storey, 2000) should be investigated in these patients as well as in ICP.

We thank J.P. Carbillet and J.M. Rebibou for their technical and clinical assistance, 5. Dalstein for statistical analysis, and J.L. Prétet for critically revising the manuscript. O. Humbey acknowledges support from the Association Régionale pour l'Enseignement et la Recherche Scientifique et Technologique (ARERS), and the Ligue Nationale contre le Cancer (Comités du Doubs et de la Haute Saône).

REFERENCES

Barba A, Tessari G, Boschiero L, Chieregato GC: Renal transplantation and skin diseases: review of the literature and results of a 5-year follow-up of 285 patients. *Nephron* 73:131–136, 1996

Bastiaens MT, Struyk L, Tjong-A-Hung SP, *et al:* Cutaneous squamous cell carcinoma and p53 codon 72 polymorphism: a need for screening? *Mol Carcinog* 30:56–61, 2001

Beckman G, Birgander R, Sjalander A, Saha N, Holmberg PA, Kivela A, Beckman L: Is p53 polymorphism maintained by natural selection? *Hum Hered* 44:266–270, 1994

Berkhout RJ, Tieben LM, Smits HL, Bavinck JN, Vermeer BJ, ter Schegget J: Nested PCR approach for detection and typing of epidermodysplasia verruciformis-associated human papillomavirus types in cutaneous cancers from renal transplant recipients. *J Clin Microbiol* 33:690–695, 1995

Blohme I, Larko O: Skin lesions in renal transplant patients after 10–23 years of immunosuppressive therapy. *Acta Dermatol Venereol (Stockh)* 70:491–494, 1990

Bouwes-Bavinck JN, Berkhout RJM: HPV infections and immunosuppression. *Clin Dermatol* 15:427–437, 1997

Bouwes Bavinck JN, Claas FHJ, Hardie DR, Green A, Vermeer BJ, Hardie IR: Relation between HLA antigens and skin cancer in renal transplant recipients in Queensland, Australia. *J Invest Dermatol* 108:708–711, 1997

Boyle J, MacKie RM, Briggs JD, Junor BJR, Aitchison TC: Cancer, warts, and sunshine in renal transplant recipients: a case-control study. *Lancet* 1:702, 1984

Burk RD, Kadish AS: Treasure hunt for human papillomaviruses in non melanoma skin cancers. *J Natl Cancer Inst* 88:781–782, 1996

Dokianakis DN, Koumantaki E, Billiri K, Spandidos DA: P53 codon 72 polymorphism as a risk factor in the development of HPV-associated non-melanoma skin cancers in immunocompetent hosts. *Int J Mol Med* 5:405–409, 2000

Ducloux D, Caron PL, Rebibou JM, *et al:* CD4 lymphocytopenia as a risk factor for skin cancers in renal transplant recipients. *Transplantation* 65:1270–1272, 1998

van Duin M, Snijders PJ, Vossen MT, *et al:* Analysis of human papillomavirus type 16, E6 variants in relation to p53 codon 72 polymorphism genotypes in cervical carcinogenesis. *J General Virol* 81:317–325, 2000

Euvrard S, Chardonnet Y, Pouteil-Noble C, Kanitakis J, Chignol MC, Thivolet J, Touraine JL: Association of skin malignancies with various and multiple carcinogenic and noncarcinogenic human papillomaviruses in renal transplant recipients. *Cancer* 72:2198–2206, 1993

Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG: A broad range of

human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. *J General Virol* 80:2437–2443, 1999

Hamel N, Black MJ, Ghadirian P, Foulkes WD: No association between p53 codon 72 polymorphism and the risk of squamous cell carcinoma of the head and neck. *Br J Cancer* 82:757–759, 2000

Hardie IR: Skin cancer in transplant recipients. *Transplant Rev* 9:1, 1995

Hildesheim A, Schiffman M, Brinton LA, *et al:* p53 polymorphism and risk of cervical cancer. *Nature* 396:531–532, 1998

Hopfl R, Bens G, Wieland U, Petter A, Zelger B, Fritsch P, Pfister H: Human papillomavirus DNA in non-melanoma skin cancers of a renal transplant recipient: detection of a new sequence related to epidermodysplasia verruciformis associated types. *J Invest Dermatol* 108:53–56, 1997

Irwin M, Marin MC, Phillips AC, *et al:* Role for the p53 homologue p73 in E2F-1-induced apoptosis. *Nature* 407:645–648, 2000

Jackson S, Storey A: E6 proteins from diverse cutaneous HPV types inhibit apoptosis in response to UV damage. *Oncogene* 19:592–598, 2000

de Jong-Tieben LM, Berkhout RJM, ter Schegget J, *et al:* The prevalence of human papillomavirus DNA in benign keratotic skin lesions of renal transplant recipients with and without a history of skin cancer is equally high: a clinical study to assess risk factors for keratotic skin lesions and skin cancers. *Transplantation* 69:44–49, 2000

Lindelöf B, Sigurgeirsson B, Gabel H, Stern RS: Incidence of skin cancer in 5356 patients following organ transplantation. *Br J Dermatol* 143:513–519, 2000

Lissy NA, Davis PK, Irwin M, Kaelin WG, Dowdy SF: A common E2F-1 and p73 pathway mediates cell death induced by TCR activation. *Nature* 407:642–645, 2000

Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM: The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 7:209–214, 1989

Marin MC, Jost CA, Brooks LA, *et al:* A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. *Nat Genet* 25:47–54, 2000

Marshall SE, Bordea C, Haldar NA, Mullighan CG, Wojnarowska F, Morris PJ, Welsh KI: Glutathione S-transferase polymorphisms and skin cancer after renal transplantation. *Kidney Int* 58:2186–2193, 2000*a*

Marshall SE, Bordea C, Wojnarowska F, Morris PJ, Welsh KI: P53 codon 72 polymorphism and susceptibility to skin cancer after renal transplantation. *Transplantation* 69:994–996, 2000*b*

Matlashewski GJ, Tuck S, Pim D, Lamb P, Schneider J, Crawford LV: Primary structure polymorphism at amino acid residue 72 of human p53. *Mol Cell Biol* 7:961–963, 1987

McGregor JM, Berkhout RJ, Rozycka M, ter Schegget J, Bouwes Bavinck JN, Brooks L, Crook T: p53 mutations implicate sunlight in post-transplant skin cancer irrespective of human papillomavirus status. *Oncogene* 15:1737–1740, 1997

O'Connor DP, Kay EW, Leader M, Atkins GJ, Murphy GM, Mabruk MJ: P53 codon 72 polymorphism and human papillomavirus associated skin cancer. *J Clin Pathol* 54:539–542, 2001

O'Nions J, Brooks LA, Sullivan A, *et al:* p73 is over-expressed in vulval cancer principally as the delta2 isoform. *Br J Cancer* 85:1551–1556, 2001

Park JS, Kim EJ, Lee JY, Sin HS, Namkoong SE, Um SJ: Functional inactivation of p73, a homolog of p53 tumor suppressor protein, by human papillomavirus E6 proteins. *Int J Cancer* 91:822–827, 2001

Quin AG, Sikkink S, Rees JL: Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. *Cancer Res* 54:4756–4759, 1994

Riethmuller D, Gay C, Bertrand X, *et al:* Genital human papillomavirus infection among women recruited for routine cervical cancer screening or for colposcopy determined by hybrid capture II and polymerase chain reaction. *Diagn Mol Pathol* 8:157–164, 1999

Scheffner M, Werness BA, Huibregste JM, Levine AJ, Howley PM: The E6 oncoprotein encoded by human papilloma virus types 16 and 18 promotes the degradation of p53. *Cell* 63:1129–1136, 1990

Shamanin V, zur Hausen H, Lavergne D, *et al:* Human papillomavirus infections in non melanoma skin cancers from renal transplant recipients and non immunosuppressed patients. *J Natl Cancer Inst* 88:802–811, 1996

Storey A, Thomas M, Kalita A, *et al:* Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* 393:229–234, 1998

Tieben LM, Berkhout RJ, Smits HL, *et al:* Detection of epidermodysplasia verruciformis-like human papillomavirus types in malignant and premalignant skin lesions of renal transplant recipients. *Br J Dermatol* 131:226–230, 1994